

WHAT IS CLAIMED IS:

1 1. A reaction mixture for producing a product saccharide, wherein the
2 reaction mixture comprises an acceptor saccharide and a first type of plant or microorganism
3 cell that produces: a) a nucleotide sugar, and b) a first recombinant glycosyltransferase that
4 catalyzes the transfer of a sugar from the nucleotide sugar to the acceptor saccharide to form
5 the product saccharide.

1 2. The reaction mixture of claim 1, wherein the cells are selected from one
2 or more of the group consisting of bacterial cells, yeast cells, fungal cells, and plant cells.

1 3. The reaction mixture of claim 1, wherein the cells are permeabilized or
2 otherwise disrupted.

1 4. The reaction mixture of claim 1, wherein the glycosyltransferase is a
2 fucosyltransferase and the nucleotide sugar is GDP-fucose.

1 5. The reaction mixture of claim 1, wherein the glycosyltransferase is a
2 sialyltransferase and the nucleotide sugar is CMP-sialic acid

1 6. The reaction mixture of claim 1, wherein nucleotide sugar is selected
2 from the group consisting of UDP-Gal, UDP-Glc, UDP-Glucuronic acid, UDP-GalNAc,
3 UDP-Galacturonic acid, GDP-mannose.

1 7. The reaction mixture of claim 1, wherein the first type of cell produces
2 the nucleotide sugar at an elevated level compared to a wild-type cell.

1 8. The reaction mixture of claim 7, wherein the elevated level of the
2 nucleotide sugar results from a deficiency in the ability of the cell to incorporate the
3 nucleotide sugar into a polysaccharide normally produced by the cell.

1 **9.** The reaction mixture of claim 7, wherein the elevated level of the
2 nucleotide sugar is at least 10% higher than the level of the nucleotide sugar produced by the
3 wild-type cell.

1 **10.** The reaction mixture of claim 9, wherein the elevated level of the
2 nucleotide sugar is at least 25% higher than the level of the nucleotide sugar produced by the
3 wild-type cell.

1 **11.** The reaction mixture of claim 1, wherein the nucleotide sugar is
2 synthesized by an enzymatic pathway that includes one or more enzymes that are expressed
3 from heterologous genes.

1 **12.** The reaction mixture of claim 11, wherein the recombinant
2 glycosyltransferase is a sialyltransferase, the nucleotide sugar is CMP-sialic acid and the
3 heterologous gene encodes CMP-sialic acid synthetase.

1 **13.** The reaction mixture of claim 12, wherein the acceptor saccharide is
2 lactose and the product saccharide is sialyllactose.

1 **14.** The reaction mixture of claim 11, wherein the recombinant
2 glycosyltransferase is a β 1,4-GalNAc transferase and the nucleotide sugar is UDP-GalNAc.

1 **15.** The reaction mixture of claim 14, wherein the acceptor is lactose and
2 the product saccharide is β 1,4-GalNAc-lactose.

1 **16.** The reaction mixture of claim 11, wherein the recombinant
2 glycosyltransferase is a galactosyltransferase and the nucleotide sugar is UDP-Gal.

1 **17.** The reaction mixture of claim 16, wherein the galactosyltransferase is
2 an α 1,3-galactosyltransferase and the product saccharide contains a terminal α 1,3-linked
3 galactose residue.

1 **18.** The reaction mixture of claim **11**, wherein the enzymatic pathway
2 comprises a full or partial sugar nucleotide regeneration cycle.

1 **19.** The reaction mixture of claim **18**, wherein the nucleotide sugar is UDP-
2 GalNAc and the sugar nucleotide regeneration cycle comprises a set of enzymes selected
3 from the group consisting of:

4 UDP-GalNAc epimerase, UDP-GlcNAc pyrophosphorylase, GlcNAc-1-
5 kinase, polyphosphate kinase and pyruvate kinase; and

6 UDP-GalNAc pyrophosphorylase, GlcNAc-1-kinase, polyphosphate
7 kinase and pyruvate kinase.

1 *Subal* **20.** ~~The reaction mixture of claim **19**, wherein the reaction mixture further~~
2 ~~comprises a second cell type that produces a nucleotide that is used as a substrate for the~~
3 ~~sugar nucleotide regeneration cycle.~~

1 **21.** The reaction mixture of claim **20**, wherein the second cell type
2 comprises an exogenous gene that encodes a nucleotide synthetase polypeptide that catalyzes
3 the synthesis of the nucleotide.

1 **22.** The reaction mixture of claim **21**, wherein the first cell type comprises
2 exogenous genes that encode a) a fusion protein that comprises a polypeptide having 3'-
3 sialyltransferase activity and a polypeptide that has CMP-sialic acid synthetase activity; and
4 b) enzymes that catalyze the synthesis of sialic acid from GlcNAc;
5 and the second cell type comprises an exogenous gene that encodes
6 CMP-synthetase.

1 **23.** The reaction mixture of claim **21**, wherein the first cell type is *E. coli*
2 and the second cell type is yeast or *Corynebacterium*.

1 **24.** The reaction mixture of claim 1, wherein the first type of cell produces a
2 second recombinant glycosyltransferase that catalyzes the transfer of a sugar from the
3 nucleotide sugar to the product saccharide to form a further glycosylated product saccharide.

1 **25.** The reaction mixture of claim 24, wherein the nucleotide sugar is UDP-
2 Gal, the first recombinant glycosyltransferase is an β 1,4-galactosyltransferase and the second
3 recombinant glycosyltransferase is an α 1,3-galactosyltransferase.

1 **26.** The reaction mixture of claim 25, wherein the acceptor saccharide is
2 Glc(R) β -O-R¹, wherein R¹ is -(CH₂)_n-COX; X is selected from the group consisting of OH,
3 OR², -NHNH₂, R is OH or NAc; R² is a hydrogen, a saccharide, an oligosaccharide or an
4 aglycon group having at least one carbon atom, and n is an integer from 2 to 18.

1 **27.** The reaction mixture of claim 25, wherein the UDP-Gal is generated by
2 enzymes that are expressed from exogenous genes that encode UDP-Gal 4' epimerase and
3 UDP-Glc pyrophosphorylase.

1 **28.** The reaction mixture of claim 1, wherein the cell further comprises: a)
2 an enzymatic system for producing at least a second nucleotide sugar, and b) at least a
3 second recombinant glycosyltransferase that catalyzes transfer of a sugar from the second
4 nucleotide sugar to the product sugar.

1 **29.** The reaction mixture of claim 28, wherein:
2 the first recombinant glycosyltransferase is a GlcNAc transferase and
3 the first nucleotide sugar is UDP-GlcNAc; and
4 the second recombinant glycosyltransferase is a galactosyltransferase
5 and the second nucleotide sugar is UDP-galactose.

1 **30.** The reaction mixture of claim 29, wherein the reaction mixture forms
2 lacto-N-neotetraose (LNnT).

1 **31.** The reaction mixture of claim **1**, wherein the reaction mixture also
2 comprises at least a second type of cell that produces a) a second nucleotide sugar, and b) a
3 second recombinant glycosyltransferase that catalyzes the transfer of the sugar from the
4 second nucleotide sugar to the product saccharide.

1 **32.** The reaction mixture of claim **31**, wherein the first glycosyltransferase
2 is a galactosyltransferase and the second glycosyltransferase is a GalNAc transferase.

1 **33.** The reaction mixture of claim **31**, wherein:
2 the first cell type comprises a recombinant β 1,4-GalNAc transferase, a
3 recombinant β 1,4-Gal transferase, UDP-GalNAc and UDP-Gal; and
4 the second cell type comprises a recombinant α 2,3-sialyltransferase and
5 CMP-sialic acid.

1 **34.** The reaction mixture of claim **33**, wherein the CMP-sialic acid is
2 produced from CTP and GlcNAc by an enzymatic system in the second cell type that
3 includes recombinant enzymes CMP-sialic acid synthetase, GlcNAc epimerase, NeuAc
4 aldolase, and CMP-synthetase.

1 **35.** The reaction mixture of claim **33**, wherein the acceptor saccharide is
2 lactosylceramide or lyso-lactosylceramide and the product saccharide is ganglioside GM₂.

1 **36.** The reaction mixture of claim **33**, wherein the second cell type further
2 comprises a recombinant α 2,8-sialyltransferase.

1 **37.** The reaction mixture of claim **36**, wherein the acceptor is
2 lactosylceramide or lyso-lactosylceramide and the product saccharide is GD₂.

1 **38.** The reaction mixture of claim 1, wherein the reaction mixture also
2 comprises a second type of cell that produces a nucleotide from which is synthesized the
3 nucleotide sugar produced by the first type of cell.

1 **39.** The reaction mixture of claim 38, wherein nucleotide produced by the
2 second cell type and the corresponding nucleotide sugar are selected from the group
3 consisting of:

4 UTP: UDP-Gal, UDP-GalNAc, UDP-GlcNAc, UDP-Glc, UDP-
5 glucuronic acid, or UDP-galacturonic acid;

6 GTP: GDP-Fuc; and

7 CTP: CMP-sialic acid.

1 ~~40.~~ A cell that produces a product saccharide, wherein the cell comprises:

2 a) a recombinant gene that encodes a glycosyltransferase;

3 b) an enzymatic system for forming a nucleotide sugar that is a
4 substrate for the glycosyltransferase; and

5 c) an exogenous saccharide acceptor moiety;

6 wherein the glycosyltransferase catalyzes the transfer of a sugar from
7 the nucleotide sugar to the acceptor moiety to produce the product saccharide.

1 **41.** The cell of claim 40, wherein the enzymatic system for forming a
2 nucleotide sugar comprises cycle enzymes for regenerating the nucleotide sugar.

1 **42.** The cell of claim 40, wherein the recombinant gene that encodes a
2 glycosyltransferase is a heterologous gene.

1 **43.** The cell of claim 40, wherein the cell forms the nucleotide sugar at an
2 elevated level compared to a wild-type cell.

1 **44.** The cell of claim **43**, wherein the elevated level of nucleotide sugar
2 results from a deficiency in the ability of the cell to incorporate the nucleotide sugar into a
3 polysaccharide normally produced by the cell.

1 **45.** The cell of claim **44**, wherein the deficiency is due to a reduced level of
2 a polysaccharide glycosyltransferase activity.

1 **46.** The cell of claim **40**, wherein the product saccharide is produced at a
2 concentration of at least about 1 mM.

1 **47.** The cell of claim **40**, wherein the enzymatic system for forming a
2 nucleotide sugar comprises an enzyme encoded by a heterologous gene.

1 *Sub 2* **48.** The cell of claim **47**, wherein the enzyme encoded by the heterologous
2 gene is one or more of:
3 a GDP-mannose dehydratase, a GDP-mannose 3,5-epimerase, and a
4 GDP-mannose 4-reductase;
5 a UDP-galactose 4' epimerase;
6 a UDP-GalNAc 4' epimerase;
7 a CMP-sialic acid synthetase;
8 a pyrophosphorylase selected from the group consisting of a UDP-Glc
9 pyrophosphorylase, a UDP-Gal pyrophosphorylase, a UDP-GalNAc pyrophosphorylase, a
10 GDP-mannose pyrophosphorylase, and a UDP-GlcNAc pyrophosphorylase;
11 a kinase selected from the group consisting of myokinase, pyruvate
12 kinase, acetyl kinase, creatine kinase; and
13 pyruvate decarboxylase.

1 **49.** The cell of claim **48**, wherein the nucleotide sugar is GDP-fucose.

1 **50.** A cell that produces a sulfated polysaccharide, the cell comprising:

2 a heterologous gene that encodes a sulfotransferase; and
3 an enzymatic system that produces PAPS.

1 51. The cell of claim 50, wherein the sulfated polysaccharide is selected
2 from the group consisting of heparin sulfate and carragenin.

1 52. The cell of claim 50, wherein the enzymatic system that produces PAPS
2 comprises one or more enzymes that are expressed from exogenous genes.

1 ~~53. A method of producing a product saccharide, the method comprising~~
2 ~~contacting a microorganism or plant cell with an acceptor saccharide, wherein the cell~~
3 ~~comprises:~~
4 ~~a) an enzymatic system for forming a nucleotide sugar; and~~
5 ~~b) a recombinant glycosyltransferase which catalyzes the transfer of a~~
6 ~~sugar from the nucleotide sugar to the acceptor saccharide to produce the product saccharide.~~

1 ~~54. The method of claim 53, wherein the glycosyltransferase is encoded by~~
2 ~~a heterologous gene.~~

1 55. The method of claim 53, wherein the glycosyltransferase is encoded by
2 a gene that is endogenous to the cell and is produced by the cell at an elevated level
3 compared to a wild-type cell.

1 56. The method of claim 53, wherein the product saccharide is produced at
2 a concentration of at least about 1 mM.

1 57. The method of claim 53, wherein the cell is permeabilized.

1 58. The method of claim 53, wherein the cell is an intact cell.

1 59. The method of claim 53, wherein the enzymatic system for forming a
2 nucleotide sugar comprises an enzyme that is encoded by a heterologous gene.

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60. The method of claim 59, wherein the enzyme encoded by the heterologous gene is one or more of:

- a GDP-mannose dehydratase, a GDP-4-keto-6-deoxy-D-mannose 3,5-epimerase, and a GDP-4-keto-6-deoxy-L-glucose 4-reductase;
- a UDP-galactose 4' epimerase;
- a UDP-GalNAc 4' epimerase;
- a CMP-sialic acid synthetase;
- a pyrophosphorylase selected from the group consisting of a UDP-Glc pyrophosphorylase, a UDP-Gal pyrophosphorylase, a UDP-GalNAc pyrophosphorylase, a GDP-mannose pyrophosphorylase, and a UDP-GlcNAc pyrophosphorylase; a kinase selected from the group consisting of myokinase, pyruvate kinase, acetyl kinase, creatine kinase; and
- pyruvate decarboxylase.

61. The method of claim **59**, wherein the enzyme for forming a nucleotide sugar and the glycosyltransferase are expressed as a fusion protein.

62. The method of claim **61**, wherein the fusion protein comprises a CMP-sialic acid synthetase activity and a sialyltransferase activity.

63. The method of claim **61**, wherein the fusion protein comprises a galactosyltransferase activity and a UDP-Gal 4' epimerase activity

64. The method of claim 61, wherein the fusion protein comprises a GalNAc transferase activity and a UDP-GlcNAc 4' epimerase activity.

65. The method of claim 53, wherein the nucleotide sugar is GDP-fucose and the glycosyltransferase is a fucosyltransferase.

1 66. The method of claim 53, wherein the cell forms the nucleotide sugar at
2 an elevated level compared to a wild-type cell.

1 67. The method of claim 66, wherein the elevated level of nucleotide sugar
2 results from a deficiency in the ability of the cell to incorporate the nucleotide sugar into a
3 polysaccharide normally produced by the cell.

1 68. The method of claim 67, wherein the deficiency is due to a reduced
2 level of a polysaccharide glycosyltransferase activity.

1 69. The method of claim 53, wherein the cell/nucleotide sugar are selected
2 from the group consisting of:

3 *Azotobacter vinelandii*/GDP-Man;
4 *Pseudomonas sp.*/UDP-Glc and GDP-Man;
5 *Rhizobium sp.*/UDP-Glc, UDP-Gal, GDP-Man;
6 *Erwinia sp.*/UDP-Gal, UDP-Glc;
7 *Escherichia sp.*/UDP-GlcNAc, UDP-Gal, CMP-NeuAc, GDP-Fuc;
8 *Klebsiella sp.*/UDP-Gal, UDP-GlcNAc, UDP-Glc, UDP-GlcNAc;
9 *Hansenula jadinii*/ GDP-Man, GDP-Fuc;
10 *Candida famata*/UDP-Glc, UDP-Gal, UDP-GlcNAc;
11 *Saccharomyces cerevisiae*/UDP-Glc, UDP-Gal, GDP-Man, GDP-
12 GlcNAc; and
13 *X. campesti*/UDP-Glc, GDP-Man.

1 70. The method of claim 53, wherein the cell is *Azotobacter vinelandii*, the
2 nucleotide sugar is GDP-mannose, the acceptor saccharide is lactose, the glycosyltransferase
3 is mannosyl transferase, and the product saccharide is mannosyl lactose.

- 1 71. The method of claim 53, wherein the cell is *E. coli*, the nucleotide sugar
2 is CMP-sialic acid, the acceptor saccharide is lactose, the glycosyltransferase is a
3 sialyltransferase, and the product saccharide is sialyllactose.

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